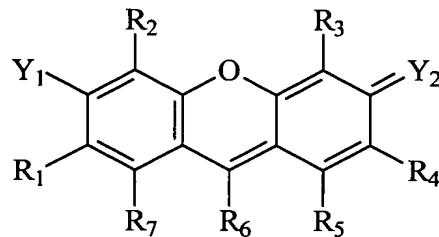


ABSTRACT

A class of aromatic-substituted xanthene compounds useful as fluorescent dyes is disclosed, the compounds having the general structure



5 where Y₁ and Y₂ taken separately are selected from the group consisting of hydroxyl, oxygen, imminium, linking group and amine, or Y₁ taken together with R₂ is cyclic imine, or Y₂ taken together with R₃ is cyclic amine; R₂, R₃, R₅, and R₇ taken separately are selected from the group consisting of hydrogen, fluorine, chlorine, lower alkyl, lower alkene, lower alkyne, sulfonate, sulfone, amino, imminium, amido, nitrile, lower alkoxy, phenyl, and linking group; R₁ taken separately is selected from the group consisting of phenyl, substituted phenyl, polycyclic aromatic, substituted polycyclic aromatic, linking group and electron-rich heterocycle, or when taken together with R₇ is selected from the group consisting of electron-rich heterocycle and indene; R₄ taken separately is selected from the group consisting of amino, amido, phenyl, substituted phenyl, polycyclic aromatic, substituted polycyclic aromatic, indene, linking group and electron-rich heterocycle, or when taken together with R₅ is selected from the group consisting of phenyl, substituted phenyl, polycyclic aromatic, substituted polycyclic aromatic, indene, and electron-rich heterocycle; and R₆ is selected from the group consisting of acetylene, lower alkyl, lower alkene, cyano, phenyl, substituted phenyl, and heterocyclic aromatic. In another aspect, the 20 invention includes methods for synthesizing the above dye compounds and intermediates. In yet another aspect, the present invention includes reagents labeled with the asymmetric benzoxanthene dye compounds, including deoxynucleotides, dideoxynucleotides, phosphoramidites, and polynucleotides. In an additional aspect, the invention includes methods utilizing such dye compounds and reagents including dideoxy polynucleotide 25 sequencing and fragment analysis methods.